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Pore Formation in a Membrane Submitted to High Voltages. Influence of the Membrane Vicosity

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We present here a new model of pore formation based on physical considerations of membrane energy. The idea is to revisit the pore expansion theory, as described by Weaver, Chizmadzhev et al. [4] in the 90's. We first recover the curvature-driven closure of the pore as obtain by Kroeger et al. [2], thanks to Langevin equation written on the pore area instead of the pore raldius. Then we consider the influence of the membrane viscosity around the region where pores have already appeared. This changes in membrane viscosity have been reported in the experiments of Tsai et al. [3] Around the pore region, the mobility of the lipids is constraint, which makes the membrane viscosity decrease locally. Then, once a critical pore radius is reached, it costs more to enlarge the pore, than to create another pore elsewhere. This has also an influence on the number of pores, which are hardly created in the high viscocity regions. Our model avoids an important drawback of the previsous models: the pore radius and the pore density cannot grow infinitely unlike the model of Krassowska, Neu et al [1]. After the derivation of the equations, we will present numerical simulations that corroborate quantitatively the experimental data obtained by patch-clamp experiments.

[1] K. DeBruin et al. Modelling electroporation in a single cell. Biophysical Journal, 77, 1999.
[2] Jens H Kroeger, et al.Curvature-driven pore growth in charged membranes during charge-pulse and voltage-clamp experiments. Biophysical journal, 96(3), 2009.
[3] J. Tsai, et al. Non-brownian diffusion of membrane molecules in nanopatterned supported lipid bilayers. Nano Letters, 8(2), 2008.
[4] J.C. Weaver and Y.A. Chimazdzhev. Theory of electroporation: A review. Bioelectrochemistry and Bioenergetics, 41, 1996.

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Phasor Plots and Spectral Phasor Analysis of Laurdan and Prodan for Membrane Heterogeneity Studies: New Frontiers in Membrane Biophysics

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Since its introduction by Weber [1], fluorophores in the PRODAN series have contributed to our understanding of hydration and packing in biological membranes. Here we apply methods based on lifetime determinations and phasor plots as well as steady-state measurements using the spectral phasor approach, for analysis of the behavior of LAURDAN and PRODAN in vesicles. The lifetime Phasor approach (Jameson et al., [2]) uses a plot of M.sin(Φ) versus M.cos(Φ), where M is the modulation ratio and Φ is the phase angle taken from frequency domain fluorometry. With Spectral phasors, introduced by Fereidouni et al [3], the steady-state fluorescence spectrum is Fourier transformed, resulting in two coordinates in x and y used for a scatter plot (Spectral phasor). The temporal Phasor approach shows significant improvement compared with older methods as regards discrimination of the effects of temperature, cholesterol content and drug addition, in our membrane model systems. This approach is very convenient for characterization of complex systems wherein lifetime heterogeneity and relaxation processes are present.

The Spectral phasor approach is a very useful method for characterization of subtle changes in membrane hydration and packing.

The major advantage of both methods is that they provide a model-less approach, which is relevant to complex studies on native systems, where endogenous fluorescence can introduce undesired mistakes. Examples of the application of both methods to membrane systems will be given.

[1] Weber et al, Biochemistry, 1979. [2] Jameson et al, Appl. Spectosc. Rev., 1991. [3] Fereidouni et al, Opt. Express, 2012.

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Scanning-Aperture Electrostatic Trapping and Manipulation of Single Nanoparticles

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Although many clever trapping methods have been devised to tame nanoparticles and macromolecules, new methods are still in great demand for handling different materials and environment. Here we develop an electrostatic trap that is created in an aqueous medium between the aperture of a nanopipet and a substrate without the need for external potentials. The scannable arrangement of a nanopipet allows us to trap, displace, or release various single particles such as gold nanoparticles and lipid vesicles at will [1]. We present our results and discuss the prospects of our work for applications in nanobiophysics and in particular for studying lipid membranes.

[1] J. T. Kim, V. Sandoghdar, *under review*.

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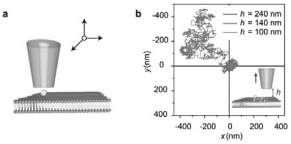

Figure -a, Schematics of the arrangement for trapping and manipulating a nanoparticle on a lipid membrane. b, Trajectories of a gold nanoparticle attached to a lipid bilayer for three different pipet heights. For h=100 and 140 nm, the particle is confined to a few tens of nanometers.

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Correlated Lateral Diffusion of Lipids

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Recent years have witnessed a proliferation of publications concerning the lateral diffusion of membrane lipids. The reported diffusion coefficient (D) values appear to depend on systems under study and on techniques of investigation. There is no clear consensus regarding their dynamics that would explain the different D values as well as the relation between mobility and the radius of the diffusing object.

Here we focus on the lateral diffusion of lipids, which has been less scrutinized than that of membrane proteins. Using fluorescence recovery after photobleaching, we have obtained lateral diffusion coefficients of lipids inserted into bilayers of giant unilamellar vesicles. We demonstrate that lipids diffuse with various diffusion coefficients ranging from D = 3.7 ± 0.4 to 13.9 ± 0.6 $\mu m^2/s$, and that these values depend on the type of lipid. Interestingly, a transmembrane peptide, having nearly the same radius as lipids and whose hydrophobic thickness matches that of the bilayer, exhibits a D value of 9.6 ± 0.4 $\mu m^2/s$. Since the lipids and the transmembrane peptide possess a similar diameter and lipids do not span membranes, the peptide diffusion coefficient is expected to be smaller than that of lipids, which is obviously not the case. Our systematic study suggests that the slower diffusion of lipids (as compared to that of the transmembrane peptide) is caused by the formation of dynamic lipid nano-patches that diffuse like a single object with an increased radius. These nano-patches seem to form more spontaneously when lipids are saturated. Consequently, one should be cautious when comparing diffusion coefficient of transmembrane proteins and lipids. Transmembrane peptides should be used as a reference instead of lipids.

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Creating Obstacle Courses for Raft Proteins - How Micropatterning Can Help Decipher Plasma Membrane Organization

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According to prevailing theory, sterol- and sphingolipid enriched nanodomains exist in the mammalian plasma membrane but are too small and dynamic to allow for proper characterization. Attempts to artificially enhance putative tendencies in the plasma membrane to create optically resolvable phase separation have yielded new model systems like Giant Plasma Membrane Vesicles, bridging the gap between synthetic lipid bilayers and live cell membranes.

In this work, we take those approaches one step further and attempt to create large scale phase separation in a live cell situation. To this end, we use a micropatterning technique to artificially create regions in the PM of live cells where we use a model raft protein glycosylphosphatidylinositol (GPI-GFP) is immobilized and highly enriched (via surface-immobilzed antibodies) at different densities to monitor the effects on plasma membrane organization and properties.

Our key findings are: 1) Within patterned regions, we can immobilize and enrich GPI-GFP up to densities of 10,000 proteins/μm2 corresponding to average center-center distances of 9nm. 2) We do not observe colocalization of raft markers (CD59, cholera toxin) within GPI-GFP patterns. 2) The diffusion of CD59 is unhindered within patterns up to GPI-GFP obstacle distances of ~17nm. At even small distances, the diffusion coefficient of CD59 decreases until a threshold is reached at ~11nm. 3) A fluorescent cholesterol derivative diffuses virtually unhindered within patterns even at GPI-GFP distances of 9-10nm. Considering the size of the GPI-GFP obstacles and Fab-labeled CD59, the slowing of diffusion within patterns can probably be explained by steric hindrance alone, arguing against the association of either GPI-GFP or CD59 in nanodomains. We further find no evidence for the formation of a continuous raft phase or even an enrichment of a different, raft-like membrane phase within patterns.